

Zika Virus Outbreak - Should assisted reproduction patients avoid pregnancy?

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ABSTRACT

Objective: To discuss the requirement from the National Health Surveillance Agency (ANVISA), for assisted reproduction treatment patients to undergo laboratory tests for ZIKV detection, and if the public health authorities and government leaders' recommendations to women simply avoid pregnancy is prudent.

Methods: This study was performed in a university-affiliated *in vitro* fertilization center in Brazil. We present a critical discussion on the risk of microcephaly due to ZIKV infection and the prevalence of other harmful pathogens to vulnerable pregnant women and infants. We assessed, 954 patients undergoing intracytoplasmic sperm injection cycles (ICSI), between April and November of 2016, concerning the results of ZIKV test, according to different regions in Brazil.

Results: Patients undergoing ICSI cycles were split into groups, according to their region of origin: 28 (3.0%) were from the North, 27 (2.8%) were from the Northeast, 40 (4.2%) were from the Midwest, 830 (87.2%) were from the Southeast, and 29 (3.0%) were from the South. Concerning the diagnosis, 112 samples had a positive or inconclusive result for ZIKV, by chromatography immunoassay. These samples were re-analyzed by ELISA and no result was positive. All positive results were from the Southeast region and none from the Northeast or Midwest regions, which are considered endemic regions.

Conclusion: ZIKV test before the onset of assisted reproduction treatments does not rule out the risk of the infection during pregnancy. In addition, although ZIKV infection risk is extremely high, the microcephaly risk due to ZIKV is not higher than the risk of miscarriage and birth defects due to other recognized pathogens.

Keywords: Zika virus, pregnancy, miscarriage, microcephaly, birth defects.

INTRODUCTION

Originally discovered in 1947, Zika virus (ZIKV), received special attention since cases of microcephaly were reported following a 2015 outbreak in Brazil (Fauci & Morens, 2016). For half a century, fewer than 20 human infections were documented (Faye *et al.*, 2014). The first reported outbreak of Zika fever occurred in 2007 on the Western Pacific island of Yap in the Federated States of Micronesia (Duffy *et al.*, 2009); this was followed by a larger epidemic in the French Polynesia, in the South Pacific in 2013 and 2014 (Cao-Lormeau *et al.*, 2014). In 2015, ZIKV emerged in the Americas, and in 2016 ZIKV infection was reported in more than 20 countries or territories in South, Central, and North America (Campos *et al.*, 2015; Zanoluca *et al.*, 2015; Enfissi *et al.*, 2016; Hennessey *et al.*, 2016).

Zika virus is predominantly spread via the *Aedes* species of mosquitos; however, other forms of transmission, including blood transfusion and probable sexual and perinatal transmission, have been reported (Lanciotti *et al.*, 2008; Besnard *et al.*, 2014; Musso *et al.*, 2014; 2015).

Because of its association with congenital defects in the form of primary or congenital microcephaly (Vargas *et al.*, 2001), as well as with paralytic neuropathy symptoms of Guillain-Barré syndrome (Willison *et al.*, 2016), the World Health Organization (WHO) has declared ZIKV to be a Public Health Emergency of International Concern on February 1, 2016 (Heymann *et al.*, 2016).

Much of the concern surrounding ZIKV has focused on the link between infection in pregnancy and fetal microcephaly, and therefore several long-standing complications for both child and the family. In fact, until May of 2016, 7,438 suspected microcephaly cases have been reported in Brazil since ZIKV emerged (1,326 confirmed/4,005 investigated) (Lessler *et al.*, 2016; Victora *et al.*, 2016). Quantifying the risk of microcephaly has been complicated by the uncertainty in the number of ZIKV affected pregnancies, owing to the large fraction of cases that are asymptomatic, a lack of consensus on the definition of microcephaly, and other infectious causes of microcephaly, such as cytomegalovirus and rubella (Lessler *et al.*, 2016).

Although a causal association of ZIKV infection with microcephaly in newborns has not been confirmed, ZIKV introduction in Northeastern Brazil is associated with an increase in congenital malformations in newborns in the same period (Brazilian Health Ministry, 2016).

In a recent statement, the WHO confirms that ZIKV is "spreading explosively" and that the associated level of concern is "extremely high". This was followed by the declaration (February 2, 2016) of public health emergency of international concern around the current outbreak. Moreover, the CDC Emergency Operations Center elevated their response to ZIKV to level 1, the highest level of activation - for only the fourth time in the history of the organization. In addition, public health authorities and government leaders in multiple countries have issued first-ever recommendations for women to simply avoid pregnancy.

Moreover, the Brazilian Ministry of Health described that cases of ZIKV infection have been confirmed by laboratorial tests throughout the whole country (Brazilian Health Ministry, 2015). Additionally, with the symptoms resulting from the infection, the presence of the virus in pregnant women has been associated with cases of microcephaly and deaths of newborns. The presumed correlation between the infection of pregnant women by ZIKV and cases of microcephaly is suggested by the neurotropic character of the virus, which was not found in other organs of the fetus (Mlakar *et al.*, 2016).

The laboratorial investigation for suspected cases of ZIKV infection includes nonspecific diagnosis and the specific diagnosis, which is based on the *in vitro* detection of viral RNA, by molecular biology techniques or by the detection of antibodies anti ZIKV, from blood samples.

Therefore, the National Health Surveillance Agency (ANVISA), Collegiate Board Resolution #72/2016, established that assisted reproduction treatment patients must undergo laboratory tests for the detection of the antibody against ZIKV (IgM), in serum or plasma sample. If the result is reactive or inconclusive, the serological test (IgM) must be repeated, within at least 30 days, or the laboratorial test by molecular biology should be performed according to the assisted reproduction center protocol. For male patients, if the result is positive, tests must be carried out in sperm samples.

If the test result is "Non-reactive", together with the absence of classical and neurological signs and symptoms of ZIKV infection, the controlled ovarian stimulation procedure can be initiated for ovum pickup.

The goal of the present study is to discuss whether the requirement of the National Health Surveillance Agency (ANVISA), to assisted reproduction treatment patients undergo laboratory tests for the detection of ZIKV makes sense, and if the recommendation of public health authorities and government leaders to women simply avoid pregnancy, is a prudent recommendation, based on the prevalence of other potentially harmful agents for pregnant women.

MATERIALS AND METHODS

This study was performed in a university-affiliated *in vitro* fertilization center in Brazil, in which the issue concerning the ZIKV infection in pregnant women is a real problem, with drastic consequences for the reproductive population and assisted reproduction centers. Considering the fast spread of ZIKV worldwide, we carry out a critical discussion on the risk of microcephaly due to ZIKV infection, and prevalence of other harmful pathogens to vulnerable pregnant women and infants.

We had 954 intracytoplasmic sperm injection cycles (ICSI), performed between April and November of 2016, evaluated concerning the results of ZIKV test, according to the region of Brazil: North, Northeast, Midwest, Southeast, and South.

The ZIKV test was performed by chromatography immunoassay (Tellmefast, Biocan®, Coquitlam BC, Canada). The test is a qualitative lateral flow immunoassay for the simultaneous detection and differentiation of IgG and IgM antibodies to ZIKV in human serum. If the test results came positive a second test by enzyme linked immunosorbent assay (ELISA, Eco Diagnóstica, Vale do Sereno, Brazil), was performed. If the ELISA test came positive a PCR would be performed.

RESULTS

When patients undergoing ZIKV tests, for ICSI cycles, were split into groups according with region of origin we observed that: 28 (3%) were from the North, 27 (2.8%) were from the Northeast, 40 (4.2%) were from the Midwest, 830 (87.2%) were from Southeast, and 29 (3.0%) were from the South.

Concerning diagnosis, 112 (11.7%) samples had a positive or inconclusive result from the chromatography immunoassay. These samples were re-analyzed using ELISA and none came back positive.

All positives results were from the Southeast region and none from the Northeast or Midwest regions, which are considered endemic regions (Table 1).

Given that other infectious agents may also be potentially harmful for pregnant women, the most prevalent diseases during pregnancy in Brazil, according to epidemiologic data, are summarized in Table 2.

DISCUSSION

Undoubtedly, ZIKV infection risk deserves special attention, however, the big question is: infection with ZIKV during pregnancy leads to higher risks than other previously known pathogens?"

Pregnant women are exposed to many infectious agents that are potentially harmful. Intrauterine bacterial infection, such as: Chlamydia trachomatis, Neisseria gonorrhea, Treponema pallidum (Syphilis); viral infections, such as: Parvovirus B19, Cytomegalovirus, Varicella zoster (chickenpox), Herpes Simplex Virus; and parasitic infections, such as: Trypanosoma cruzi (Chagas disease), Toxoplasma gondii, and Plasmodium falciparum (Malaria), may represent major risks for pregnant women and fetus.

Investigators studying the 2013-2014 ZIKV outbreak in the French Polynesia estimated that the risk of microcephaly due to ZIKV infection in the first trimester of pregnancy was 0.95% and recently, the magnitude of the risk of microcephaly in Brazil is estimated to be approximately 0.88% (Johansson *et al.*, 2016).

In fact, the microcephaly risk due to ZIKV infection is extremely high; however, it is not higher than the risk of other recognized pathogens.

Another important question raised here is about the ZIKV test usefulness for assisted reproduction patients, and whether this may avoid the microcephaly due to ZIKV infection. Our findings demonstrated that the ZIKV infection in patients undergoing assisted reproduction treatments in a center in the Southeast is null, even when performed in patients from endemic regions (Midwest and the Northeast). This shows that during this short period of time, between the test diagnosis and beginning of treatment, the patient may not be infected; however, how to ensure that the infection will not occur in the following months?

Table 1. Distribution of ZIKV testes by Chromatography immunoassay and ELISA in the five Brazilian regions.

Region	N	%	Chromatography immunoassay test	ELISA test
North	28	3.0	0	0
Northeast	27	2.8	0	0
Midwest	40	4.2	0	0
Southeast	830	87.0	112	0
South	29	3.0	0	0
Total	954	100	112	0

Table 2. The prevalence of bacterial, viral and parasitic infections during pregnancy.

Type	Pathogens	Prevalence	Reference
Bacteria	<i>Chlamydia trachomatis</i>	2.57%	(Mylonas, 2012)
	<i>Neisseria gonorrhea</i>	0.7% to 7%,	(Rao <i>et al.</i> , 2008)
	Neisseria gonorrhea (risk group)	15 - 35%	(Benzaken <i>et al.</i> , 2006)
	<i>Treponema pallidum</i>	0.71%	(Gottlieb <i>et al.</i> , 2008)
	Herpes Simplex Virus	2%	(Brown <i>et al.</i> , 2005)
Parasites	<i>Trypanosoma cruzi</i>	1% to 40%	(Martins-Melo <i>et al.</i> , 2014)
	<i>Toxoplasma gondii</i>	8% - 22%	(Hill & Dubey, 2016)
	<i>Plasmodium falciparum</i>	2.9%	(Kourtis <i>et al.</i> , 2014)
Virus	Parvovirus B19 (endemic period)	1.5%	(Valeur-Jensen <i>et al.</i> , 1999)
	Parvovirus B19 (epidemic period)	13.0%	(Valeur-Jensen <i>et al.</i> , 1999)
	Cytomegalovirus	0.3%	(Preece <i>et al.</i> , 1986)
	<i>Varicella Zoster</i>	0.16% - 0.46%	(Helmuth <i>et al.</i> , 2015)
	ZIKV	0.88%	(Johansson <i>et al.</i> , 2016)

On the other hand, those patients who are already experiencing stress arising from the diagnosis of infertility and the decision to undergo an assisted reproduction treatment, also suffer from the stress of performing the ZIKV test. The instruction to perform the ZIKV associated with the alarming news coming from the media makes these patients even more frightened and stressed.

In conclusion, we believe that ZIKV test before the beginning of assisted reproduction treatments, as established by the National Health Surveillance Agency (ANVISA), Collegiate Board Resolution #72/2016, does not rule out the risk of such infection during pregnancy. In addition, although ZIKV infection risk is extremely high, especially in endemic regions, the microcephaly risk due to ZIKV is not higher than the risk of miscarriage and birth defects due to other recognized pathogens. Therefore, it is prudent to take precautions to avoid ZIKV as any other infection during pregnancy, and more importantly, pregnant women or those who wish to become pregnant naturally or through assisted reproductive techniques, should stay away from alarming news that takes away the peace of their pregnancies.

CONFLICT OF INTERESTS

The authors have no conflict of interests to report.

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